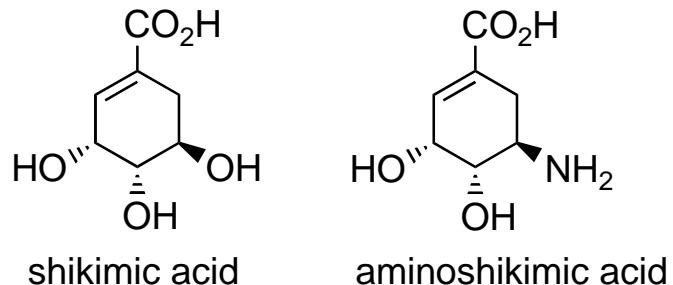
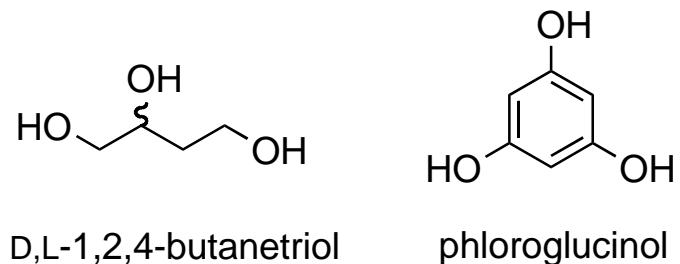


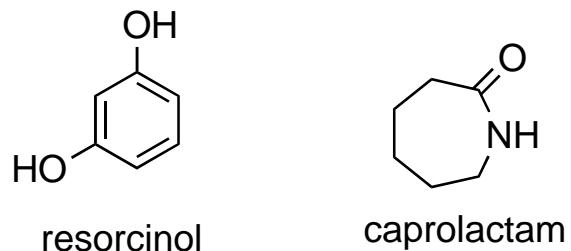
## Chiral Chemicals

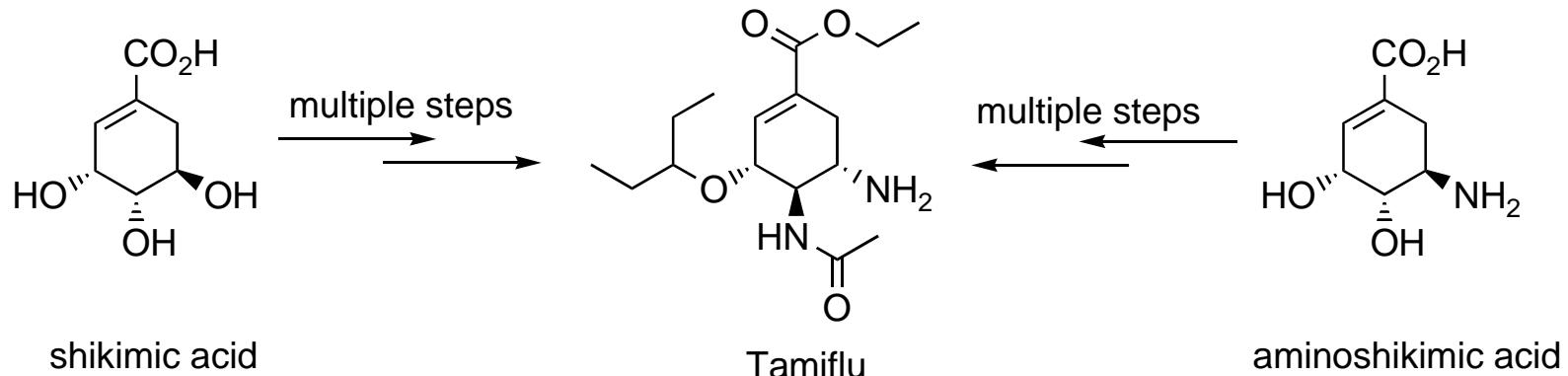


## Oxygen-Rich Chemicals

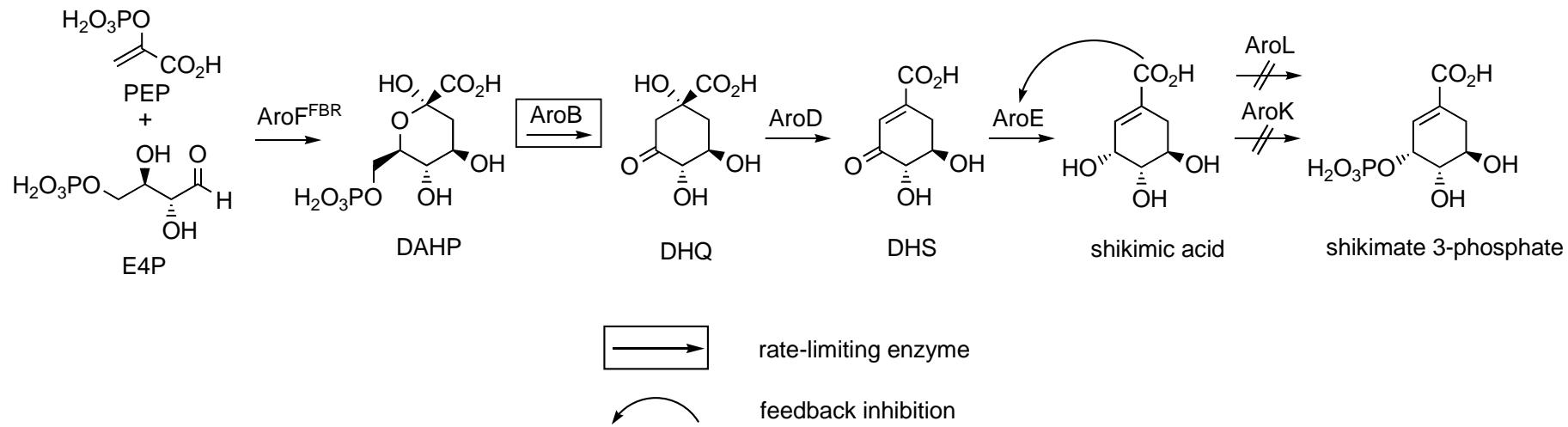


## Petrochemicals





- decreased recovery time
- reduced mortality
- prophylaxis
- early intervention

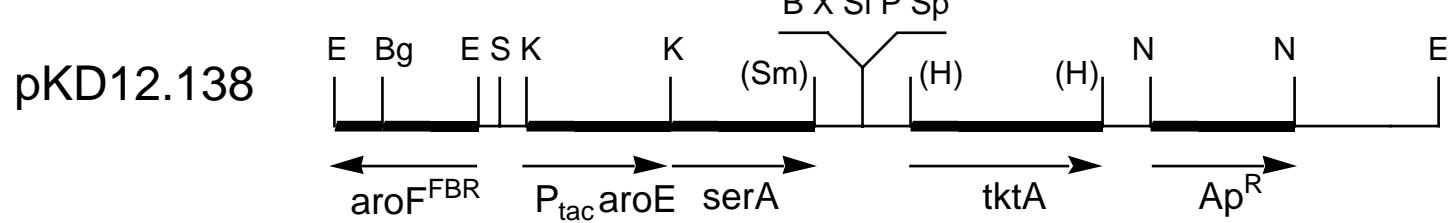


**Design Rule #1** Feedback inhibition of DAHP synthase (AroF) is the most important control of carbon flow directed into the shikimate pathway:  
Ogino, T.; Garner, C.; Markley, J. L.; Herrmann, K. M. *Proc. Natl. Acad. Sci. USA* **1982**, 79, 5828-5832.

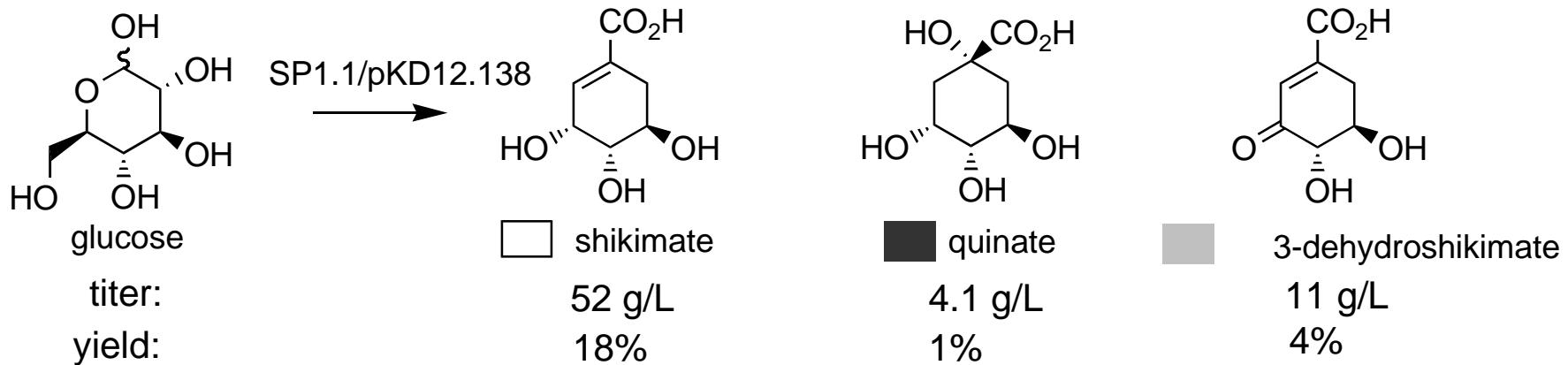
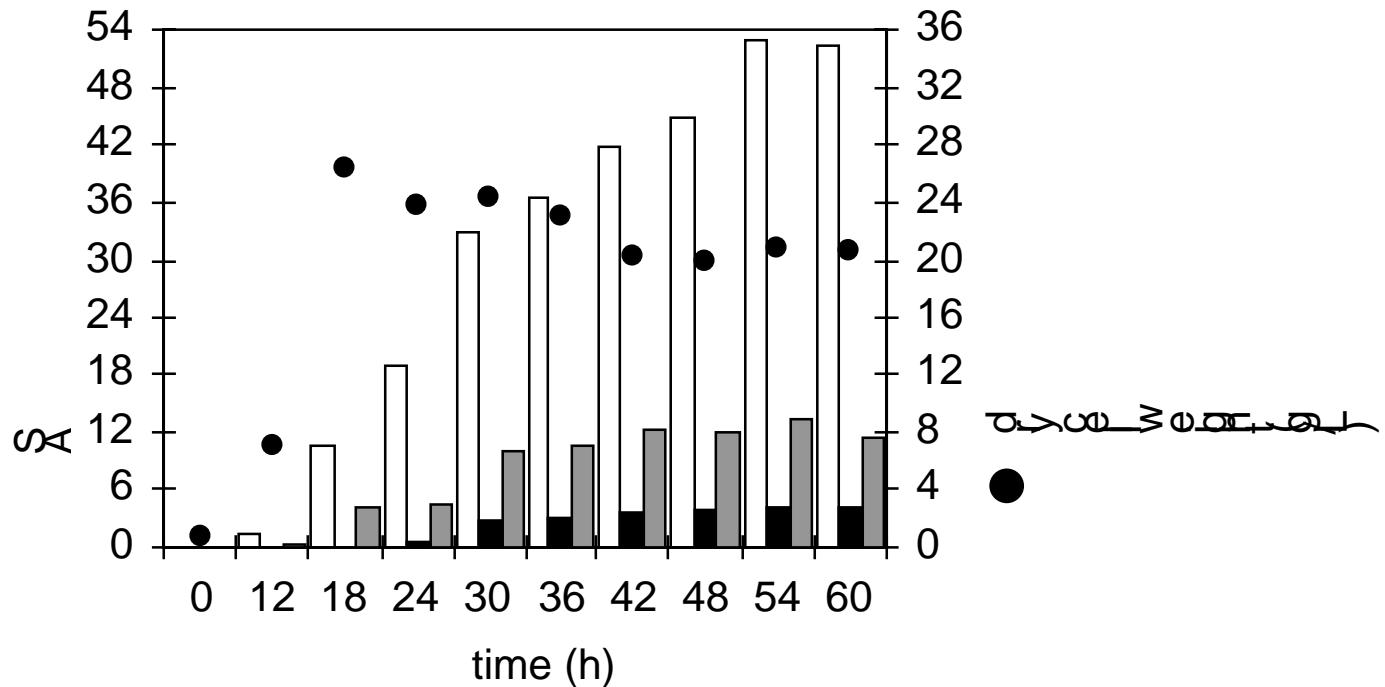
**Design Rule #2** The in vivo activity of DAHP synthase (AroF) is limited by the availability of D-erythrose 4-phosphate:  
Draths, K. M.; Pompliano, D. L.; Conley, D. L.; Frost, J. W.; Berry, A.; Disbrow, G. L.; Staversky, R. J.; Lievense, J. *J. Am. Chem. Soc.* **1992**, 114, 3956-3962.

**Design Rule #3** DHQ synthase (AroB) and shikimate dehydrogenase (AroE) impede carbon flow directed into the shikimate pathway:  
Snell, K. D.; Draths, K. M.; Frost, J. W. *J. Am. Chem. Soc.* **1996**, 118, 5605-5614.

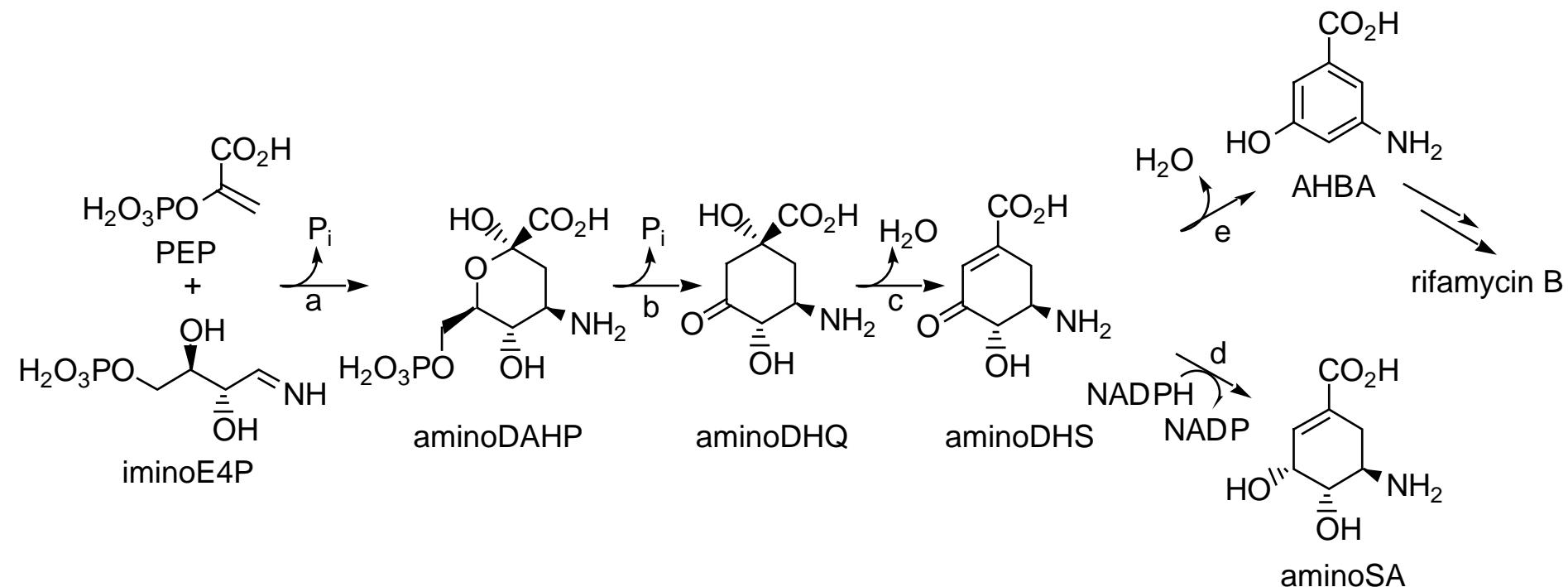
*E. coli* SP1.1    *serA ::aroB aroL::Tn10 aroK::Cm<sup>R</sup>*



# Glucose-Rich Culture of SP1.1/pKD12.138



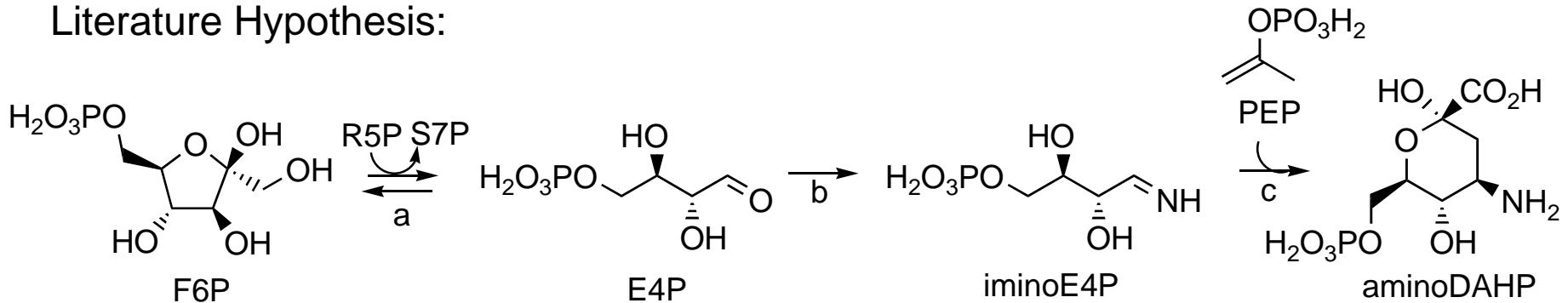
## The Aminoshikimate Pathway



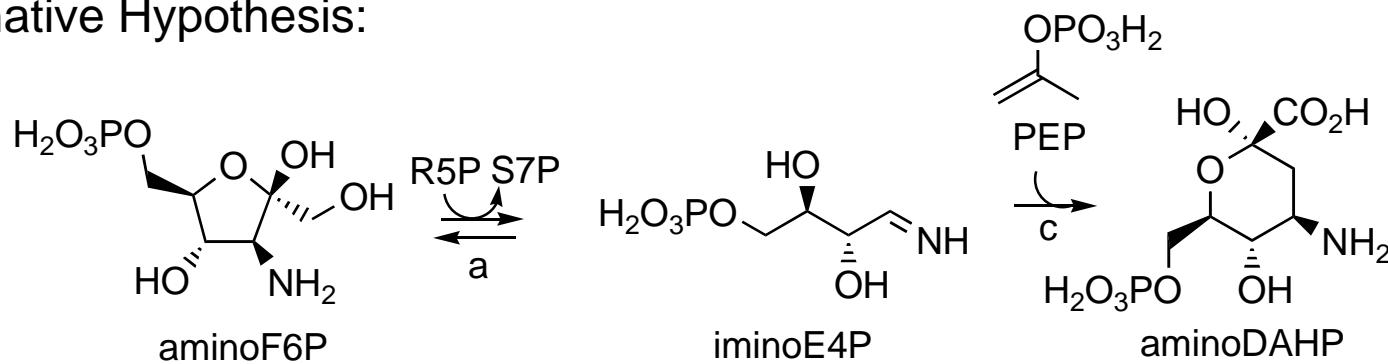
(a) *rifH*, aminoDAHP synthase; (b) *rifG*, aminoDHQ synthase; (c) *rifJ*, aminoDHQ dehydratase; (d) *rifI*, aminoquinate dehydrogenase; (e) *rifK*, AHBA synthase.

Kim, C.-G.; Kirschning, A.; Bergon, P.; Ahn, Y.; Wang, J. J.; Shibuya, M.; Floss, H. G. *J. Am. Chem. Soc.* **1992**, *114*, 4941.

Literature Hypothesis:

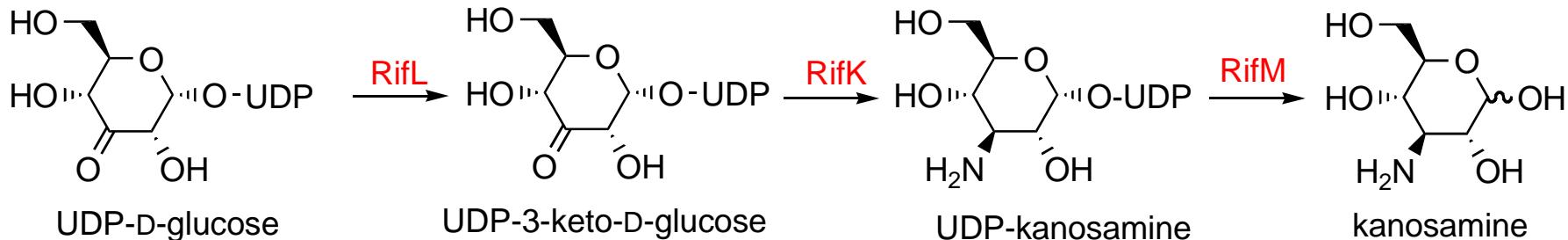


Alternative Hypothesis:

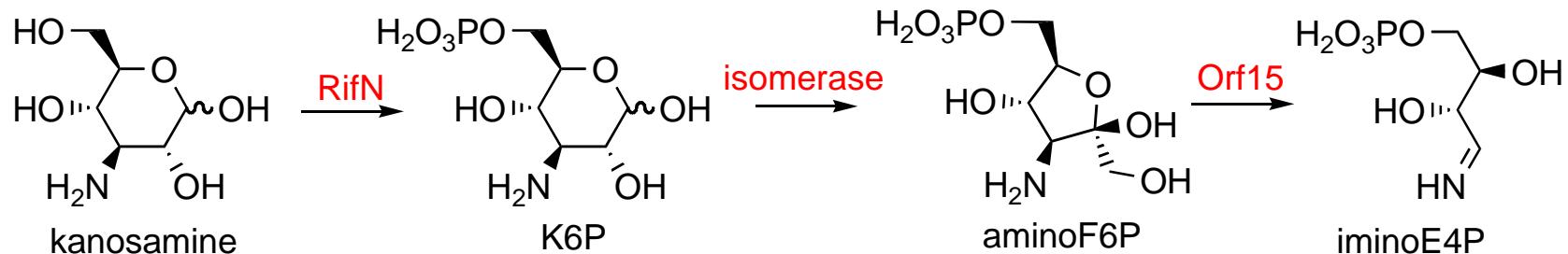


(a) transketolase, (b) transaminase, (c) aminoDAHP synthase

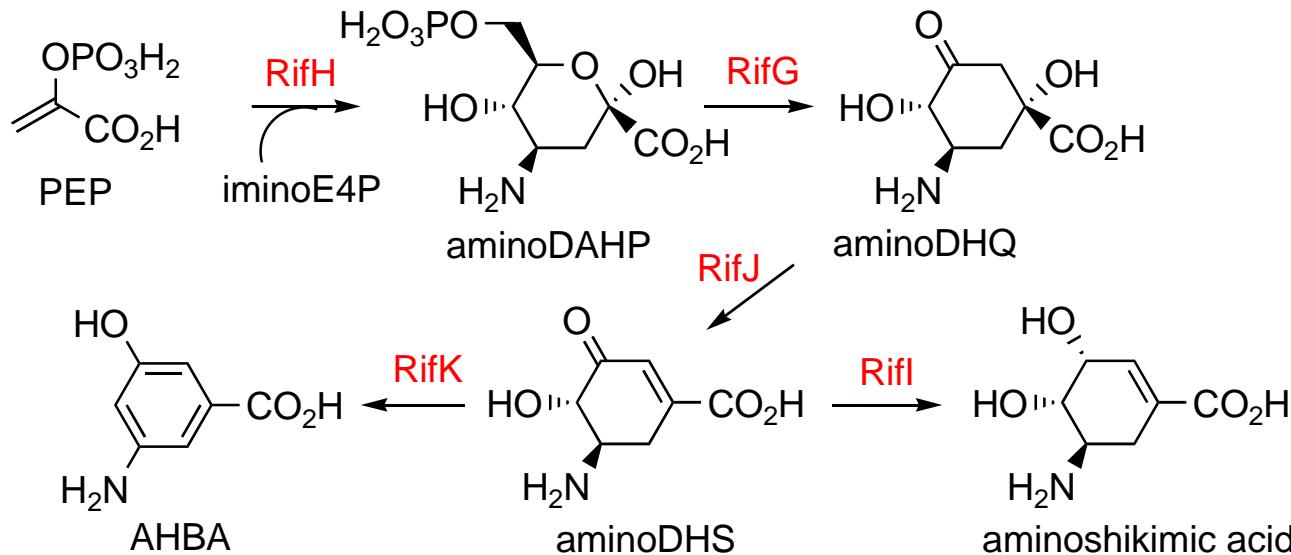
### Kanosamine Biosynthesis

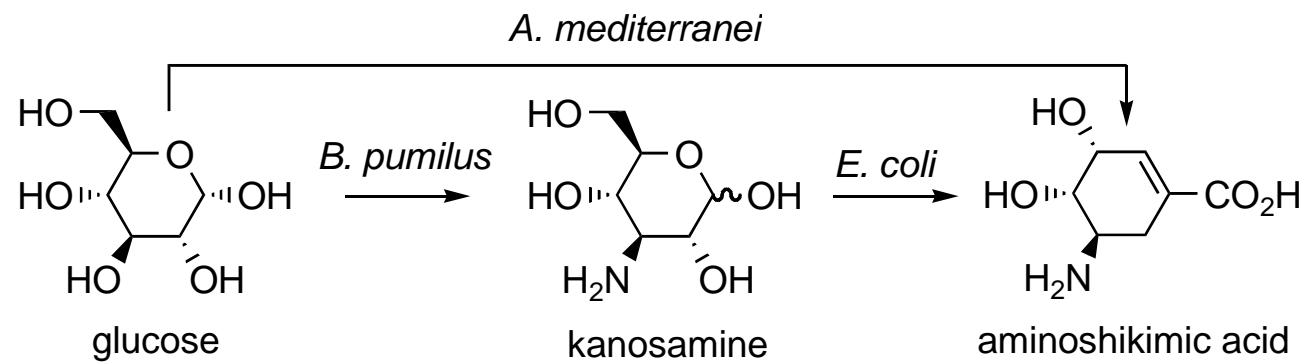


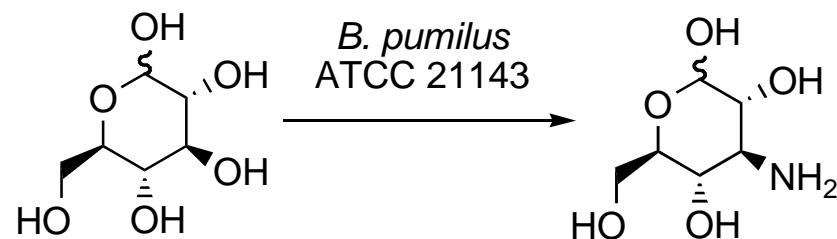
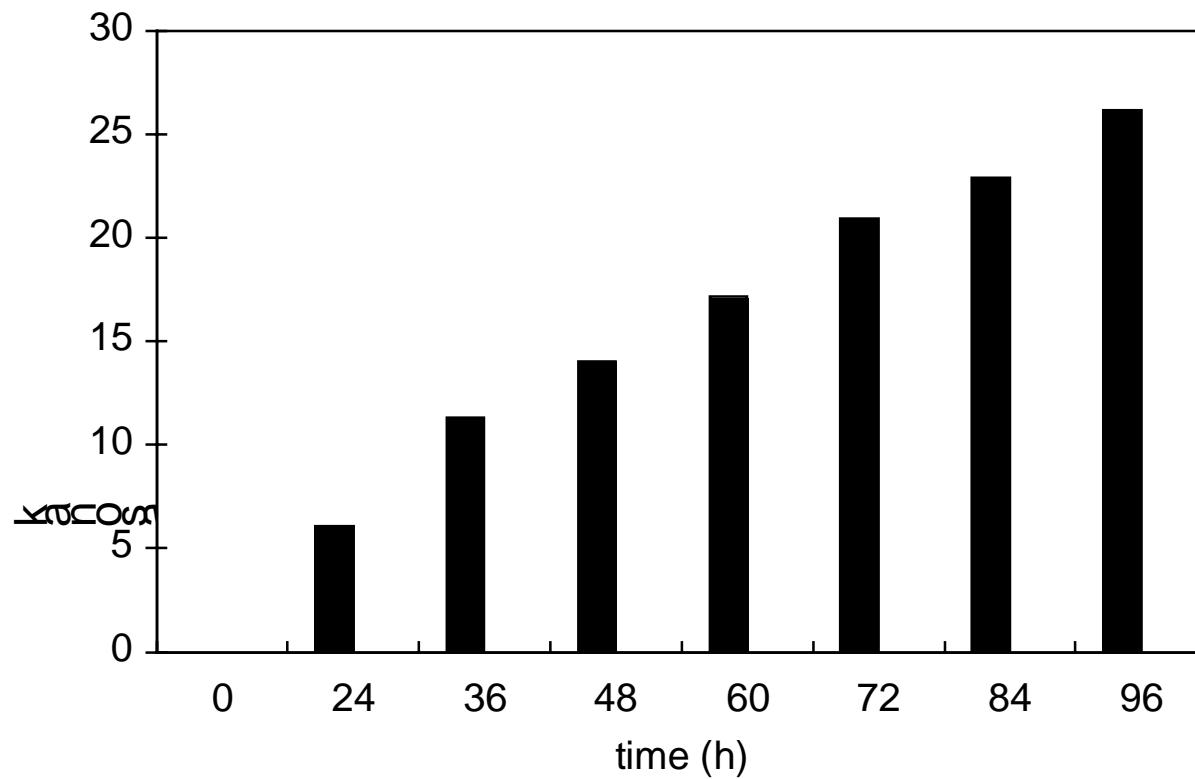
### IminoE4P Biosynthesis



### Aminoshikimate Pathway

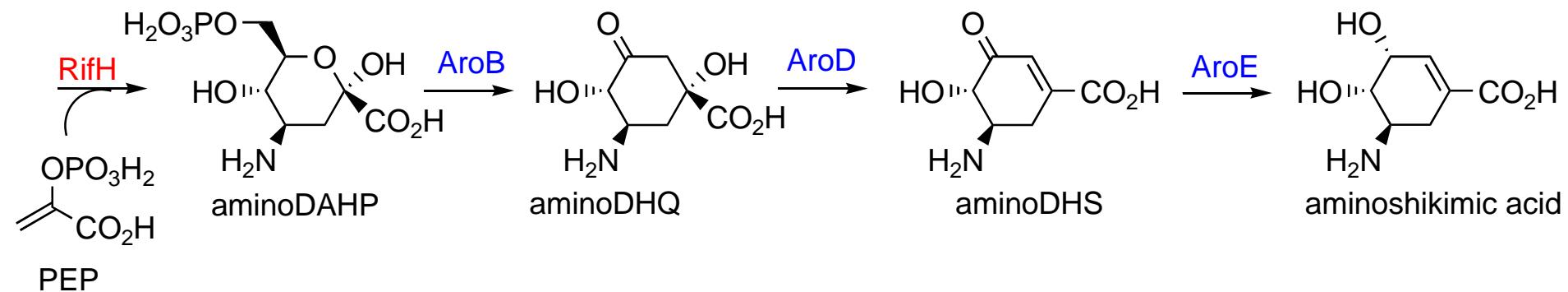
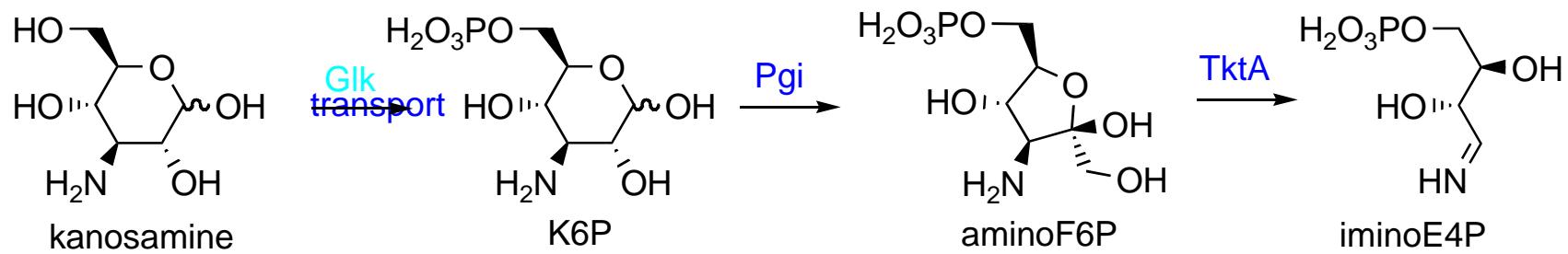






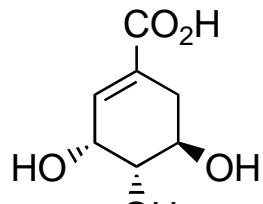
## Constructs Used for the Synthesis of Aminoshikimic Acid

entry	catalysts	plasmid characteristics	SA (g/L)	aminoSA (g/L)	aminoSA (% yield)
1	SP1.1/pJG6.223B	$P_{tac}rifH$ , $P_{tac}aroE$ , $serA$ , $tktA$ , $P_{tac}rifN$	6.4	0.24	4
2	SP1.1/pJG6.238A	$P_{tac}rifH$ , $P_{tac}aroE$ , $serA$ , $lacI^q$ , $P_{tac}orf15$ , $P_{tac}rifN$	6.3	0.4	6
3	SP1.1/pJG6.181B	$P_{tac}rifH$ , $P_{tac}aroE$ , $serA$ , $tktA$ , $P_{tac}glk$	3.4	1.1	19
4	SP1.1/pJG9.240	$P_{tac}rifH$ , $P_{tac}aroE$ , $serA$ , $lacI^q$ , $P_{tac}orf15$ , $P_{tac}glk$	7.0	1.2	19

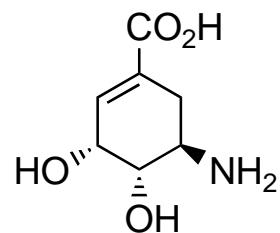




## Chiral Chemicals

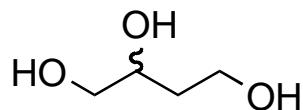


shikimic acid

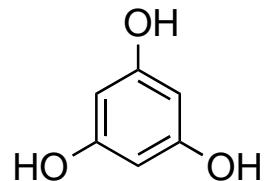


aminoshikimic acid

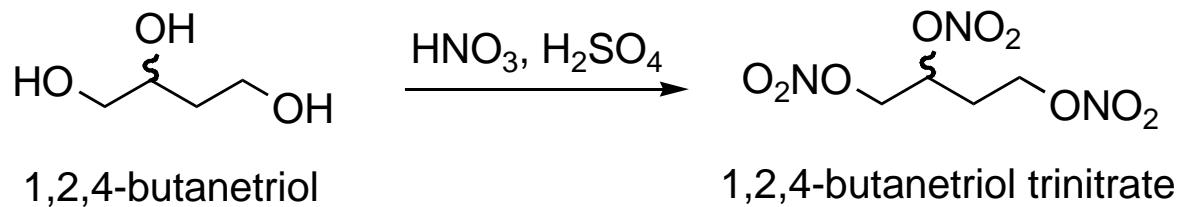
## Oxygen-Rich Chemicals



D,L-1,2,4-butanetriol

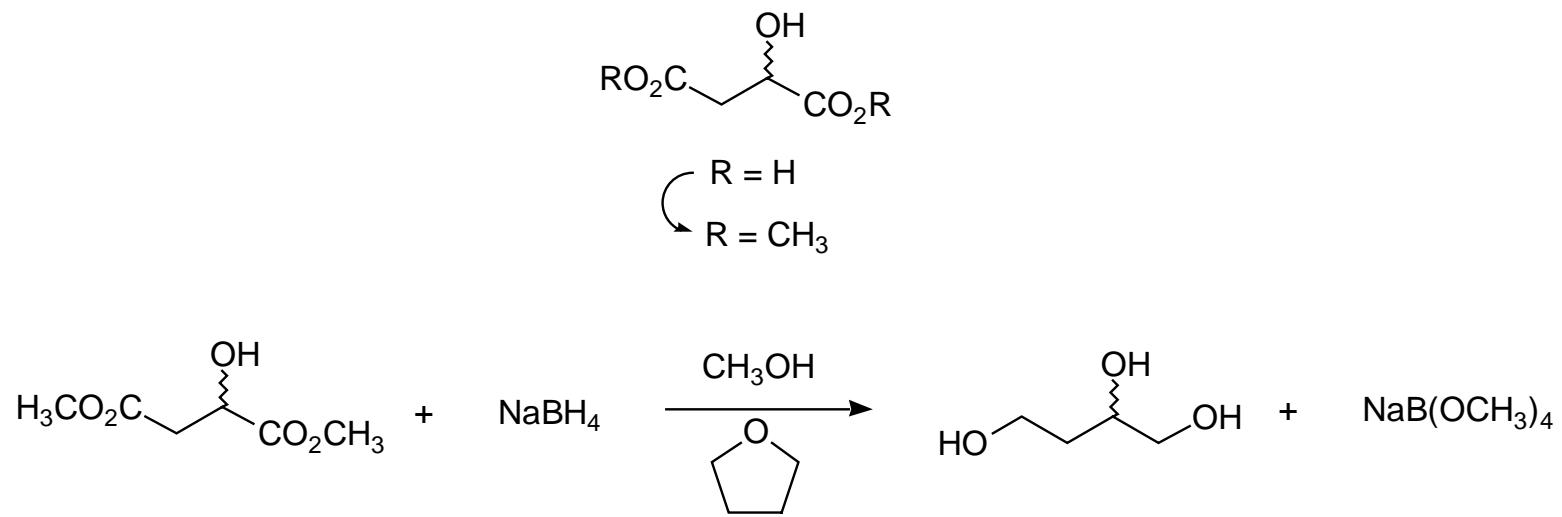


phloroglucinol



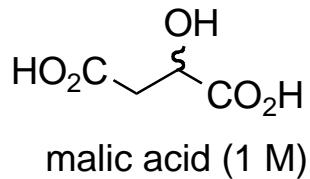
- Butanetrioltrinitrate (BTTN) is less volatile, more thermally stable, and less shock sensitive than nitroglycerine (NG).
- Replacement of NG is not currently possible due to the expense of 1,2,4-butanetriol (BT) used as the starting material for the manufacture of BTTN.

## Current Commercial BT Synthesis



2-6 tons of borate salts generated as byproduct for every ton of BT produced (WO 98/08793).

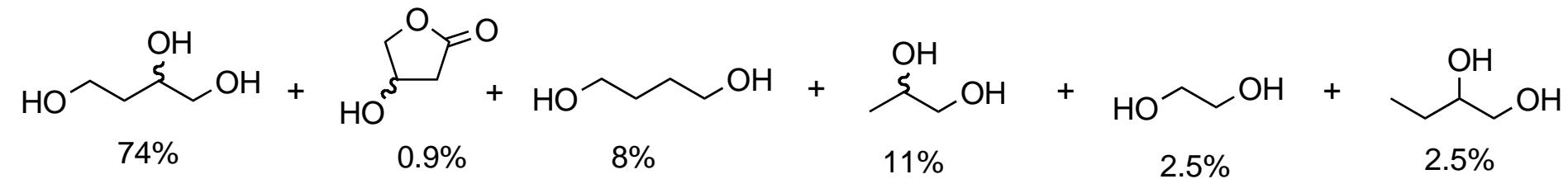
## Chemical Catalytic Alternative for BT Synthesis



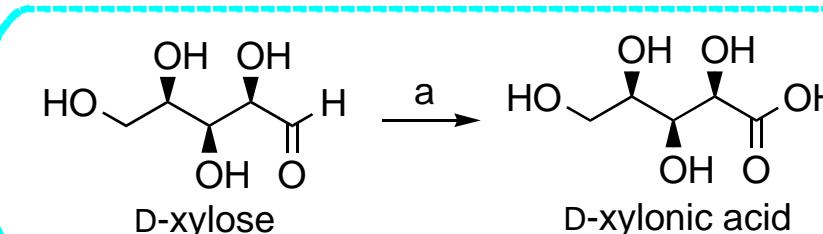
$\text{H}_2\text{O}$   
1.3 mol% of 5% Ru on C  
170 rpm

↓

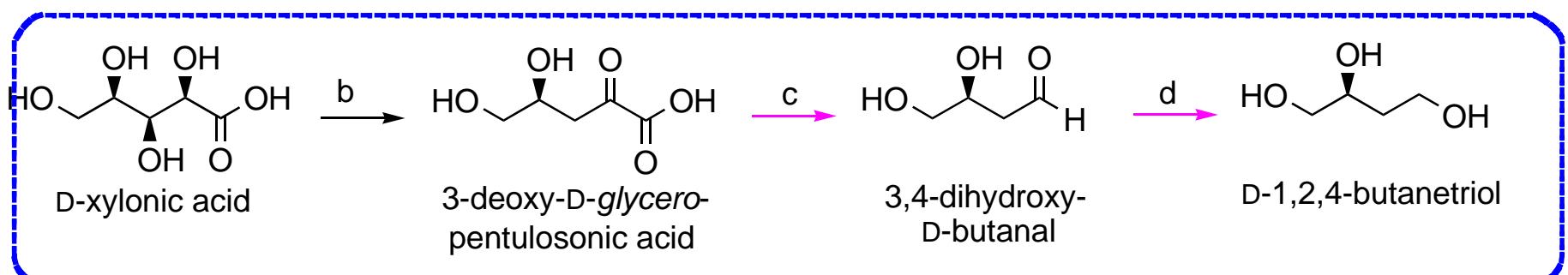
5000 psi  $\text{H}_2$   
135 °C



## Microbial Synthesis of D-BT



*Pseudomonas fragi* ATCC 4973 70% Yield



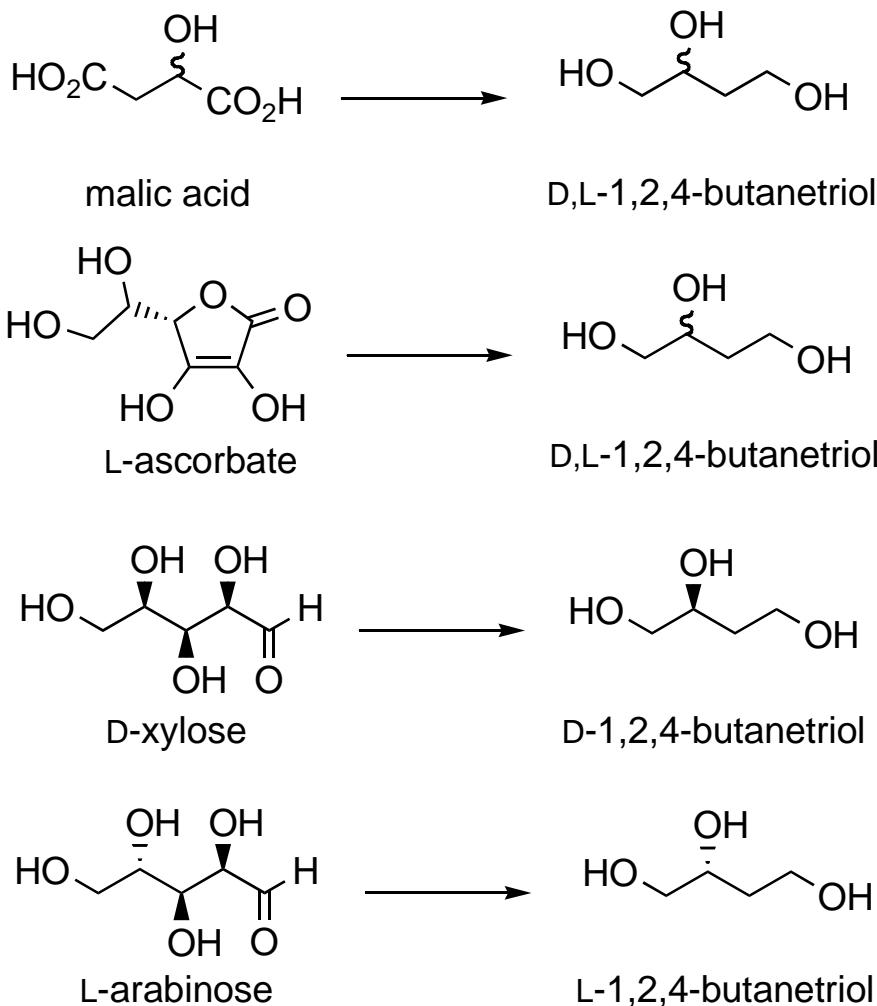
*E. coli* DH5 $\alpha$ /pWN6.186A 25% Yield

- a. D-xylonate dehydrogenase (*P. fragi*); b. D-xylonate dehydratase (*E. coli*);
- c. benzoylformate decarboxylase (*P. putida*); d. alcohol dehydrogenase (*E. coli*)

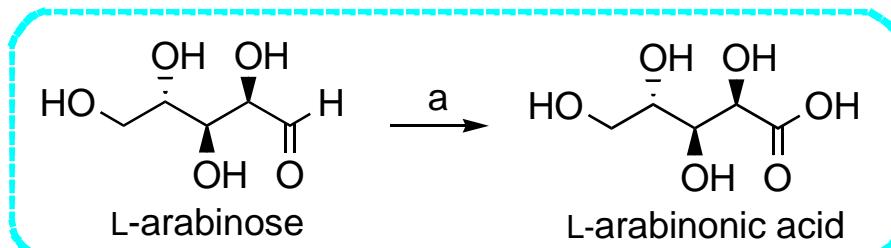
→ enzymes catalyzing reactions on native substrates

→ enzymes catalyzing reactions on non-native substrates

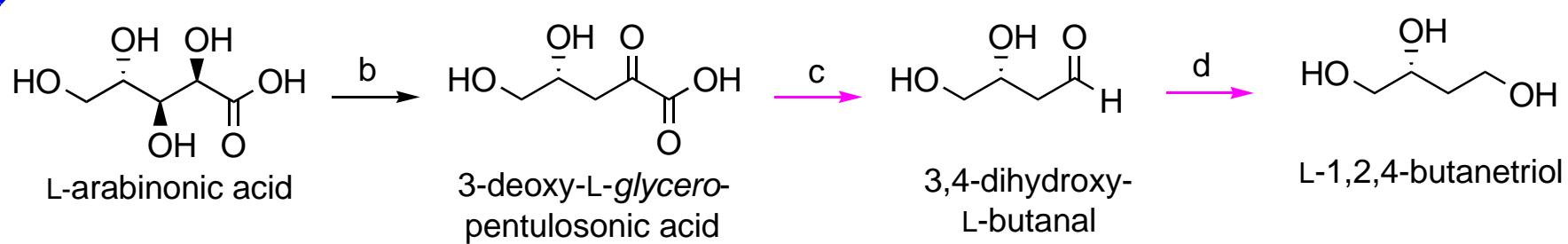
# Stereogenic Considerations



## Microbial Synthesis of L-BT



*Pseudomonas fragi* ATCC 4973 54% Yield



*E. coli* BL21(DE3)/pWN6.222A 35% Yield

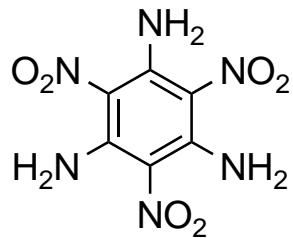
- a. L-arabinonate dehydrogenase (*P. fragi*); b. L-arabinonate dehydratase (*P. fragi*);
- c. benzoylformate decarboxylase (*P. putida*); d. alcohol dehydrogenase (*E. coli*)



enzymes catalyzing reactions on native substrates



enzymes catalyzing reactions on non-native substrates

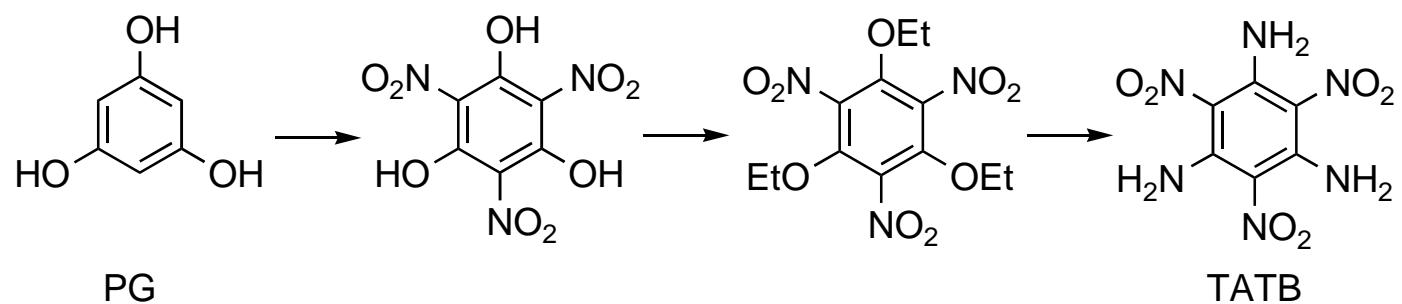


1,3,5-triamino-2,4,6-trinitrobenzene

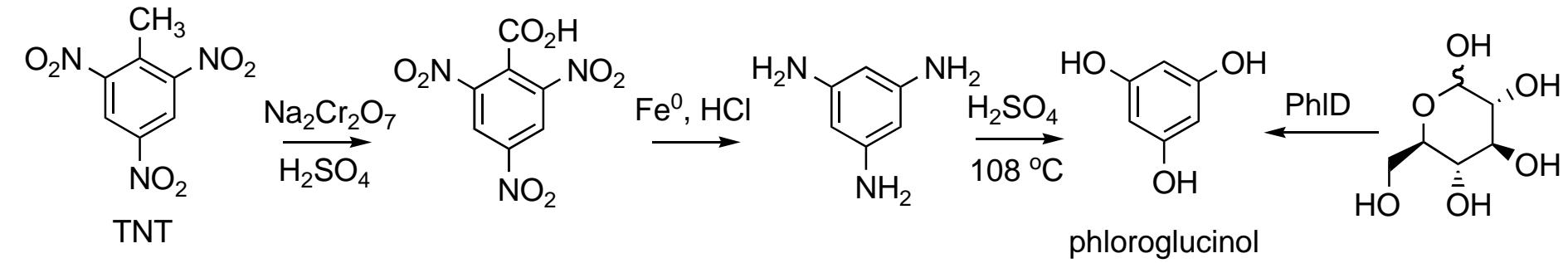
TATB

- thermally stable energetic material used in fuze and booster systems requiring high-energy, insensitive energetic compositions.
- possesses a greater density and detonation velocity relative to 2,4,6-trinitrotoluene (TNT)
- characterized by a greatly reduced shock sensitivity and increased thermal stability over hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX).

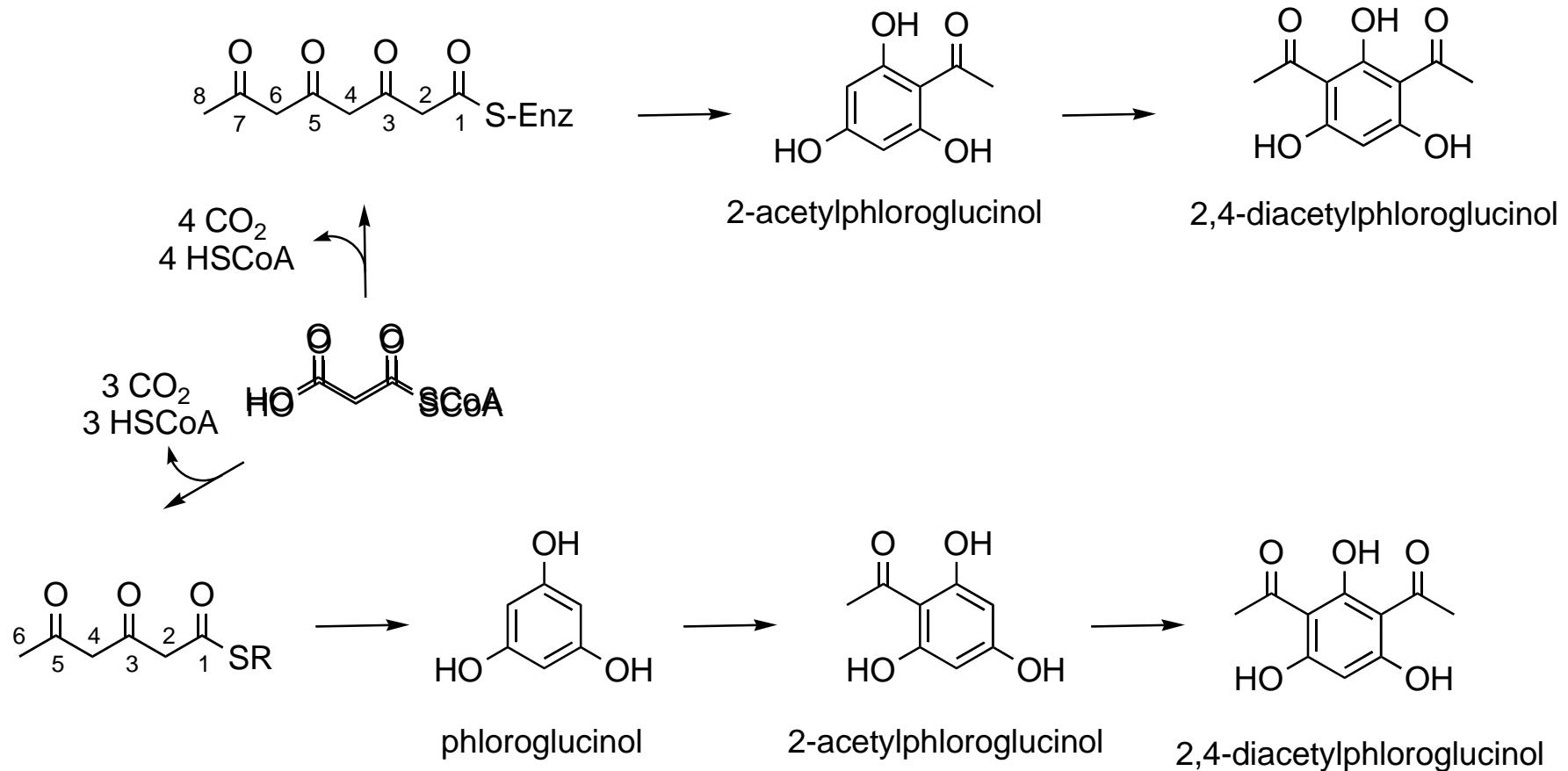
## ATK Thiokol TATB Synthesis



overall yield: 83%

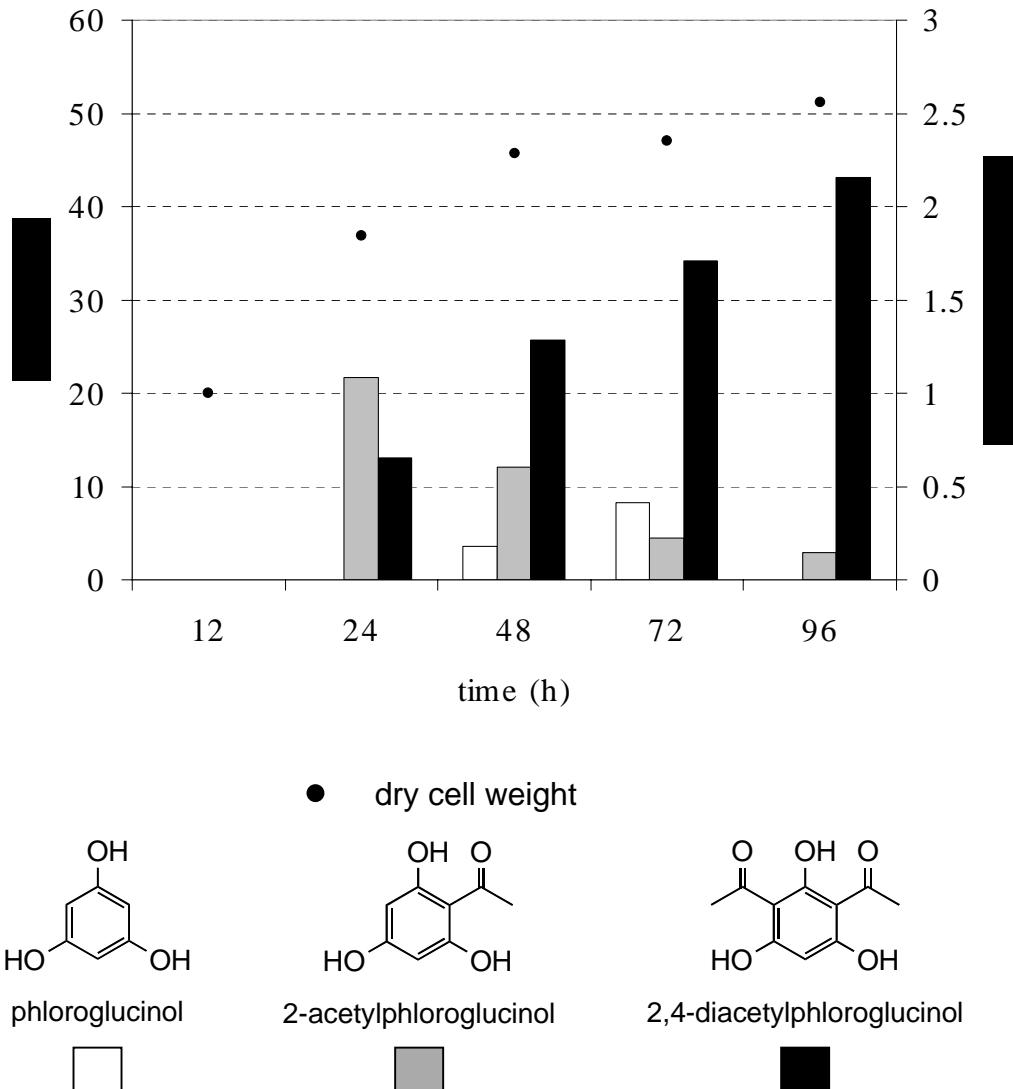


# Biosynthesis of Acetylated Phloroglucinols in *P. fluorescens* Q2-87



Bangera, M.G.; Thomashow, L.S. *J. Bacteriol.* **1999**, *181*, 3155–3163.

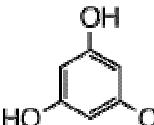
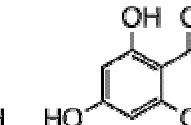
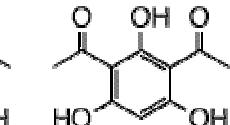
# Biosynthesis of Phloroglucinol in Wild-Type *P. fluorescens* Pf-5



Cells were grown under shake-flask conditions in YM broth (1 L); product concentrations were determined by gas chromatography after derivatization with bis(trimethylsilyl)trifluoroacetamide.

Plasmid (size)	Plasmid Map
pME6031 (8.3-kb)	<p>Plasmid map of pME6031 (8.3-kb). The map shows restriction sites Sa, St, and T<sub>T4</sub>. It includes a Tet<sup>A</sup> gene with a Tet<sup>R</sup> cassette, a Multiple Cloning Site (MCS), and the T<sub>T4</sub> promoter.</p>
pJA2.232 (15-kb)	<p>Plasmid map of pJA2.232 (15-kb). The map shows restriction sites Sa, St, S, K, and T<sub>T4</sub>. It includes a Tet<sup>A</sup> gene with a Tet<sup>R</sup> cassette, and genes phlA, phlC, phlB, phlD, and phlE under the control of P<sub>phl</sub>.</p>
pJA3.085 (11-kb)	<p>Plasmid map of pJA3.085 (11-kb). The map shows restriction sites D, Bg, N, S, and T<sub>T7</sub>. It includes an Ap<sup>R</sup> gene, the lacI gene, and genes phlA, phlC, phlB, phlD, and phlE under the control of P<sub>T7</sub>.</p>
pJA2.042 (6.5-kb)	<p>Plasmid map of pJA2.042 (6.5-kb). The map shows restriction sites D, Bg, B, and T<sub>T7</sub>. It includes a Kan<sup>R</sup> gene, the lacI gene, and the phlD gene under the control of P<sub>T7</sub>.</p>
pJA3.131A (8.1-kb)	<p>Plasmid map of pJA3.131A (8.1-kb). The map shows restriction sites D, (Bg), (Bg), B, B, and T<sub>T7</sub>. It includes a Kan<sup>R</sup> gene, the lacI gene, the serA gene, and the phlD gene under the control of P<sub>T7</sub>.</p>

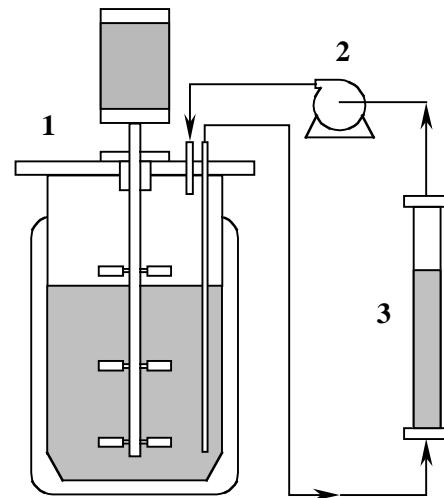
# Phloroglucinols Synthesized by Constructs Expressing *phlACBDE*

entry	construct	inserts			
1	<i>P. fluorescens</i> Pf-5/pME6031 <sup>a</sup>	(none)	10	23	35
2	<i>P. fluorescens</i> Pf-5/pJA2.232 <sup>a</sup>	<i>phlACBDE</i>	470	500	790
3	<i>E. coli</i> BL21(DE3)/pJA3.085 <sup>b</sup>	<i>phlACBDE</i>	32	14	0
4	<i>E. coli</i> BL21(DE3)/pJA3.156 <sup>b</sup>	<i>phlACBD</i>	22	13	0
5	<i>E. coli</i> BL21(DE3)/pJA2.042 <sup>b</sup>	<i>phlD</i>	<b>720</b>	0	0
6	<i>E. coli</i> BL21(DE3)/pJA3.169 <sup>b</sup>	<i>phlACB</i>	0	0	0

<sup>a</sup> Cells were grown under shake-flask conditions in YM broth (1 L).

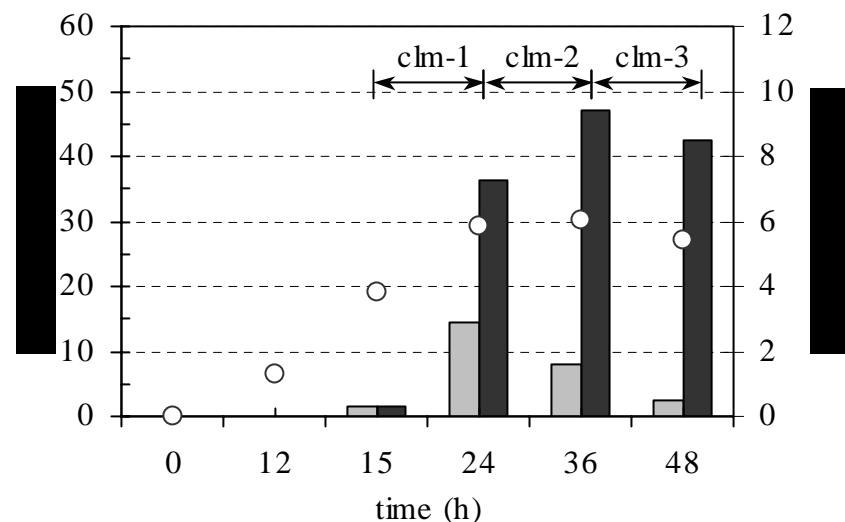
<sup>b</sup> Cells were grown under shake-flask conditions in TB medium (0.5 L), harvested and resuspended/cultured in minimal M9/glucose medium.

# In Situ, Resin-Based Extraction



1. fermentor
2. peristaltic pump
3. resin column

# Fermentor-Controlled Cultivation of JWF1(DE3)/pJA3.131A

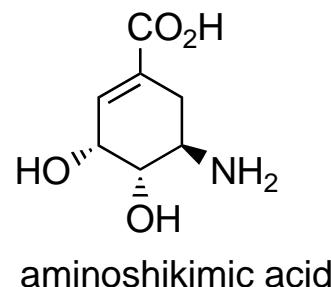
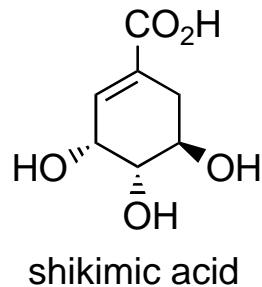


culture medium

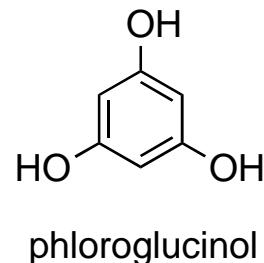
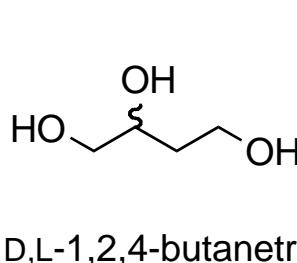


culture medium + resin-bound

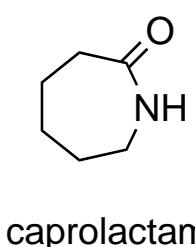
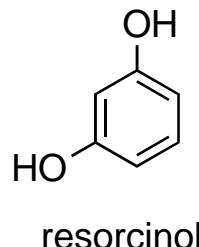
✓ Chiral Chemicals

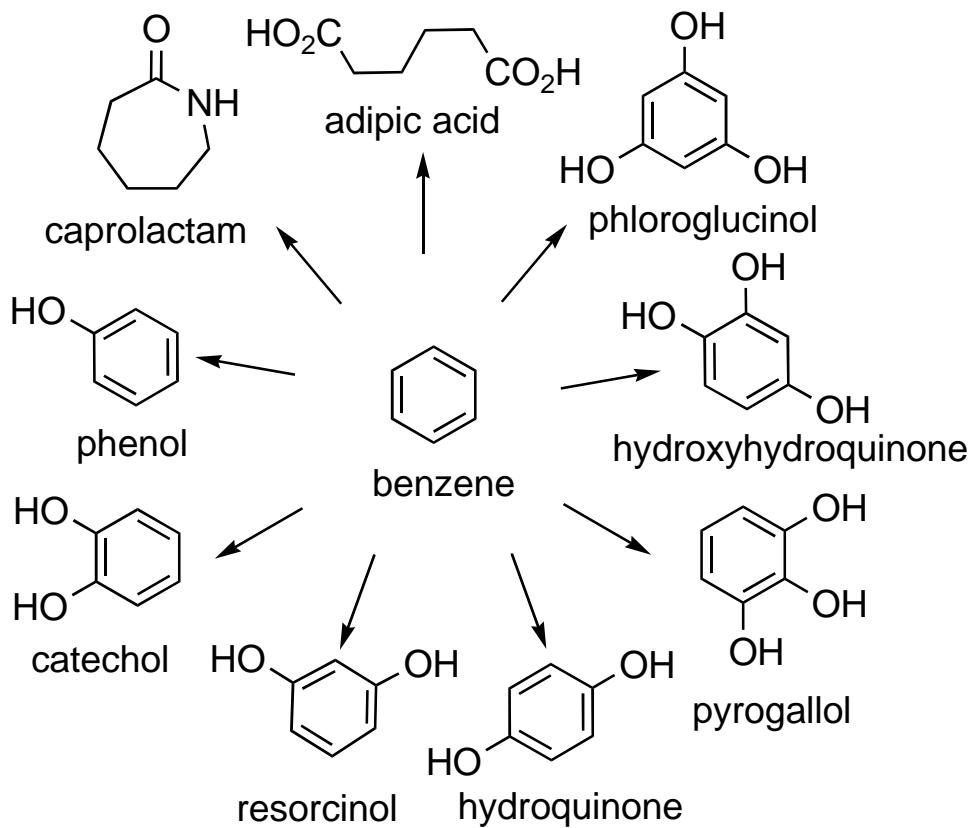


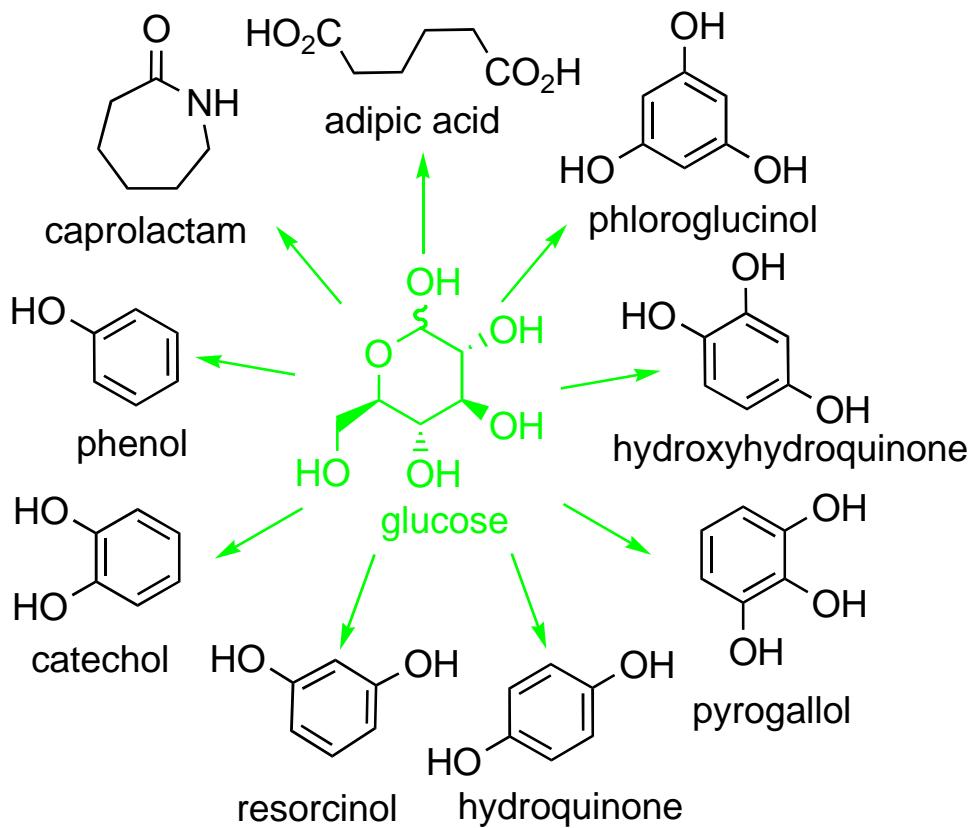
✓ Oxygen-Rich Chemicals

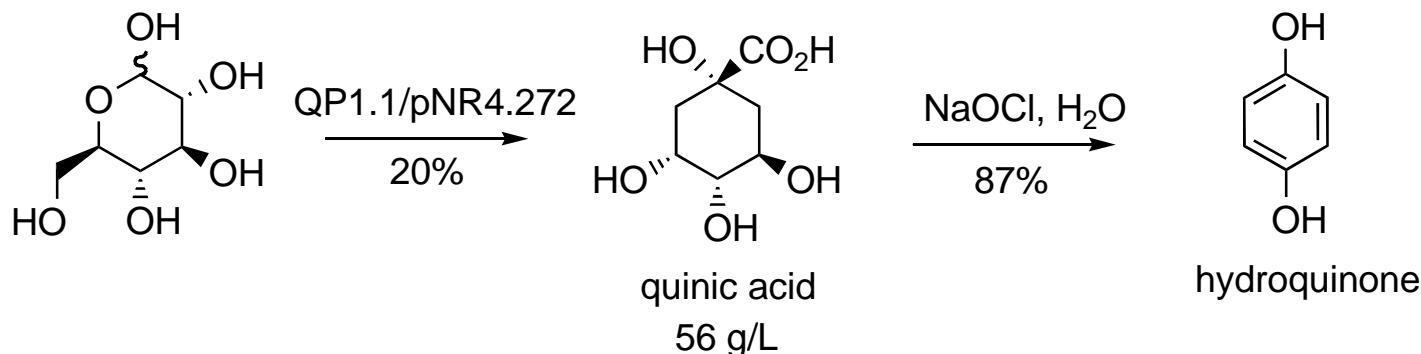


Petrochemicals



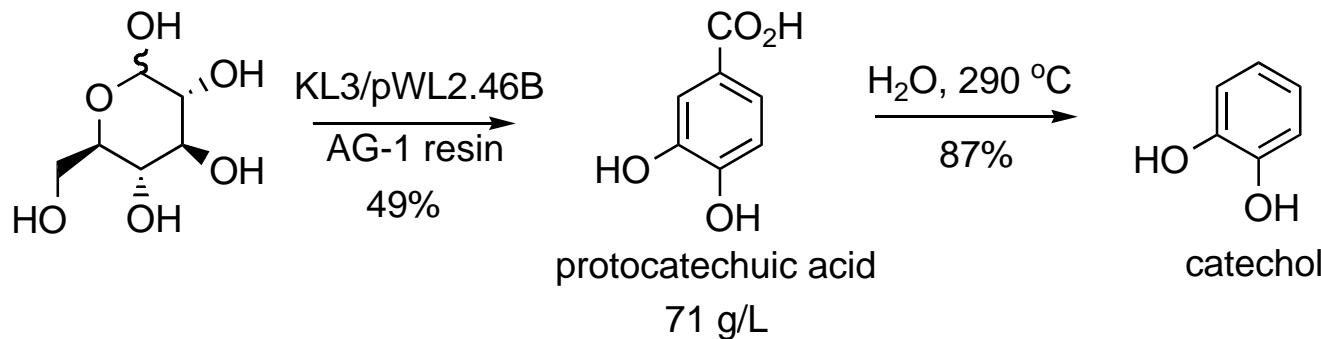






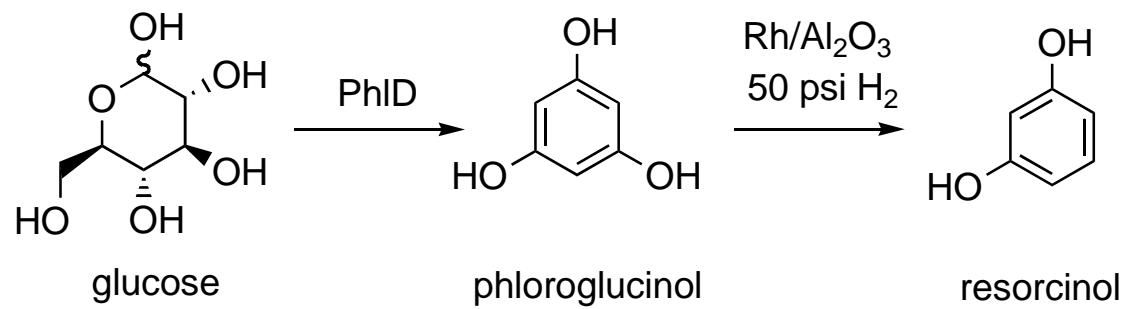
### “Benzene-Free Synthesis of Hydroquinone”

Ran, N.; Knop, D. R.; Draths, K. M.; Frost, J. W. *J. Am. Chem. Soc.* **2001**, 123, 10927.

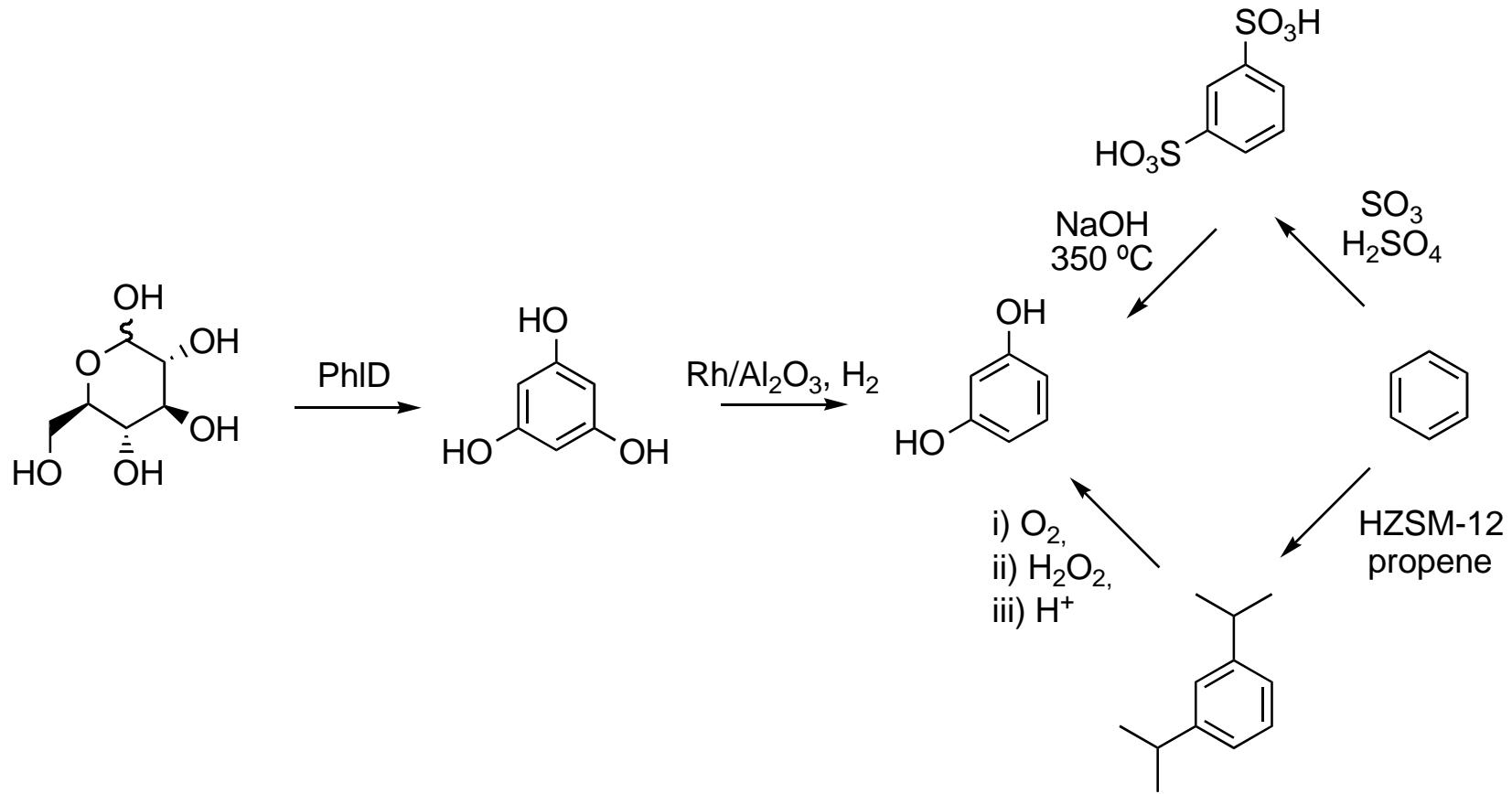


### Benzene-Free Synthesis of Catechol: Interfacing Microbial and Chemical Catalysis”

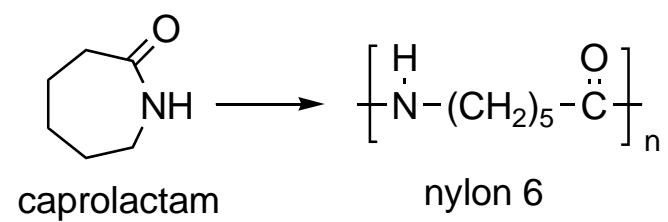
Li, W.; Xie, D.; Frost, J. W. *J. Am. Chem. Soc.* **2005**, In press.



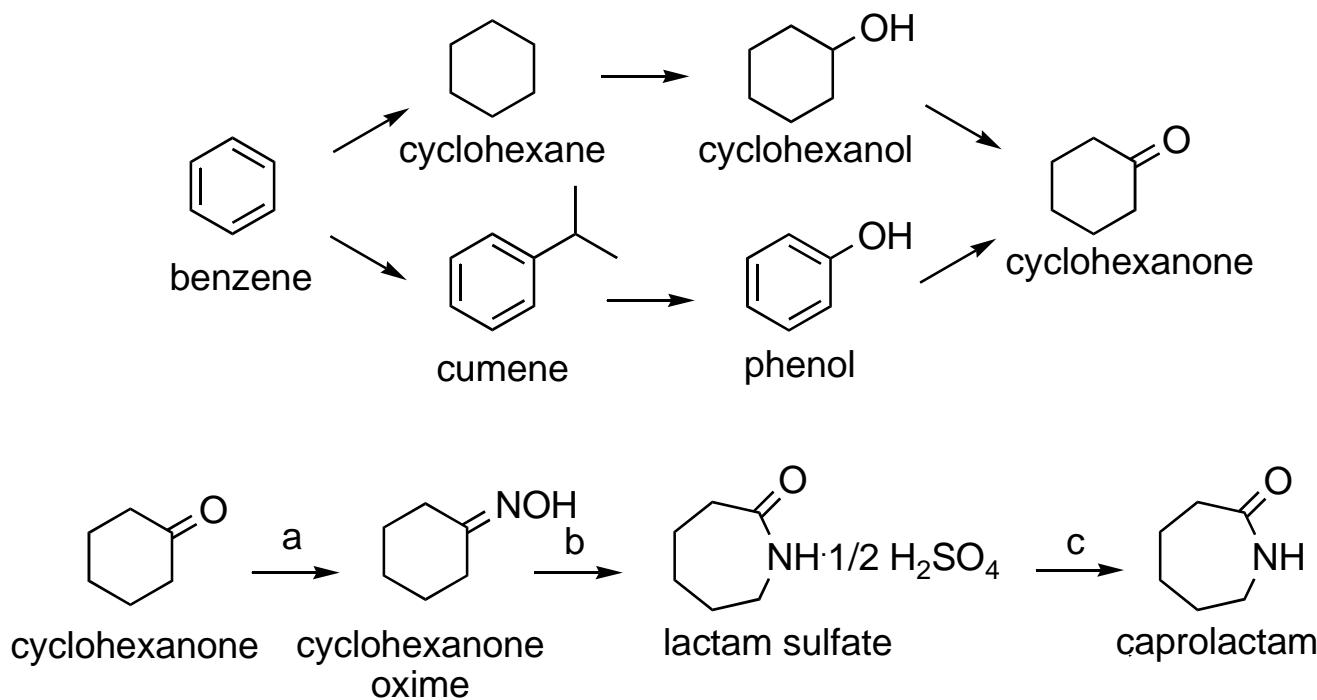
Hansen, C.; Frost, J. W. *J. Am. Chem. Soc.* **2002**, 124, 5926.



## Nylon 6 Manufacture

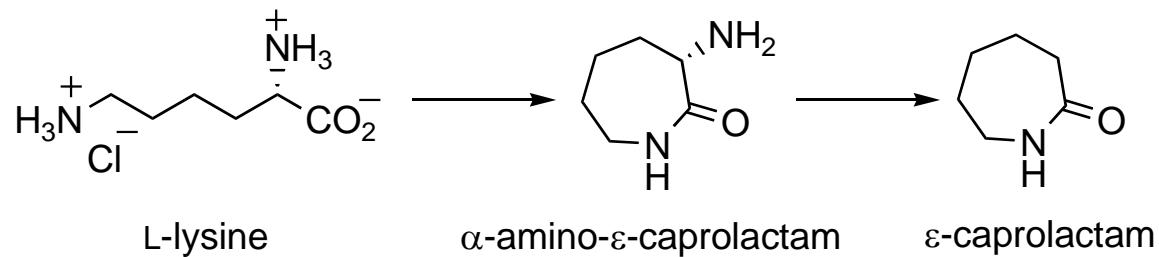


# Caprolactam Manufacture



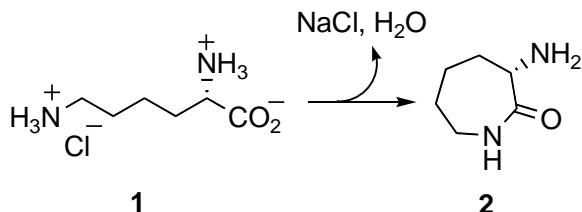
(a) i)  $(\text{NH}_2\text{OH})_2\text{H}_2\text{SO}_4$ , ii)  $\text{NH}_3$ ; (b)  $\text{H}_2\text{SO}_4$   $\text{SO}_3$ ; (c)  $\text{NH}_3$

## Biobased Caprolactam



	annual global production	price
$\epsilon$ -caprolactam	$3.6 \times 10^9 \text{ kg}$	\$0.77/lb
L-lysine	$7.5 \times 10^8 \text{ kg}$	\$0.60-1.10/lb

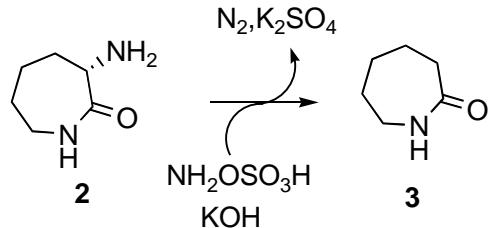
# Cyclization of L-Lysine



entry	1/NaOH/ $\text{Al}_2\text{O}_3$ (mmol)	reaction conditions	2 (% yield <sup>a,b</sup> )
1	30/30/270	1-pentanol (120 mL) 137 °C, reflux, 4 h	96 <sup>a</sup>
2	30/30/0	1-pentanol (120 mL) 137 °C, reflux, 60 h	93 <sup>a</sup>
3	30/30/0	1-hexanol (120 mL) 157 °C, reflux, 8 h	89 <sup>a</sup>
4	300/300/0	1-hexanol (1.2 L) 157 °C, reflux, 8 h	91 <sup>a</sup> /75 <sup>b</sup>
5	300/300/0	1,2-propanediol (1.2 L) 187 °C, reflux, 2 h	96 <sup>a</sup> /74 <sup>b</sup>

<sup>a</sup> Crude  $^1\text{H}$  NMR product yields were determined relative to a calibration curve based on the ratios of integrated resonances at  $\delta$  4.29 for varying concentrations of  $\alpha$ -amino- $\epsilon$ -caprolactam **2** relative to the integrated resonance at  $\delta$ =0.00 for sodium 3-(trimethylsilyl)propionate-2,2,3,3- $d_4$  in  $\text{D}_2\text{O}$ . <sup>b</sup> Yield of product purified by crystallization.

# Deamination of $\alpha$ -Amino- $\epsilon$ -Caprolactam

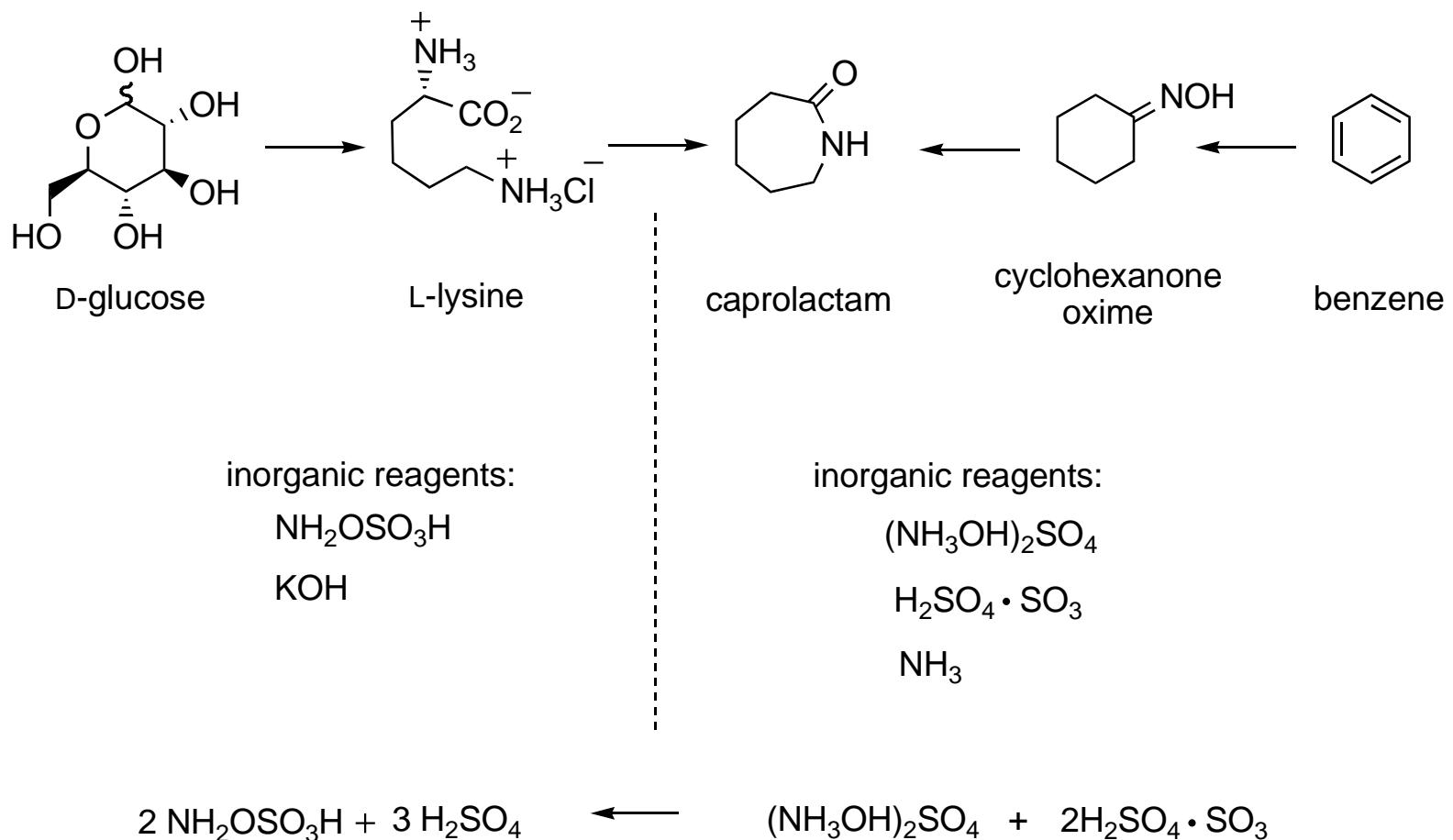


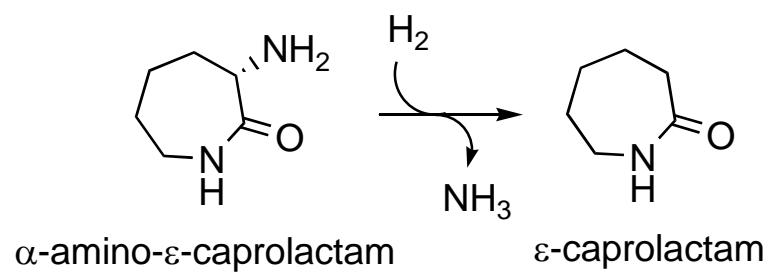
entry	<b>2</b> /KOH/ $\text{NH}_2\text{OSO}_3\text{H}$ (mmol)	$\text{H}_2\text{O}/\text{MeOH}/\text{EtOH}$ (mL)	<b>3</b> yield (%) <sup>a,b</sup>
1	20/800/400	240/160/0	61
2	20/800/400	120/80/0	62
3	20/800/400	60/40/0	64
4	20/160/80	60/40/0	65
5	20/160/80	60/0/40	70
6	20/160/80	100/0/0	75

<sup>a</sup> Product yields are after purification by sublimation. <sup>b</sup> Yields are based on L-lysine starting material. Intermediate  $\alpha$ -amino- $\epsilon$ -caprolactam was not purified prior to deamination.

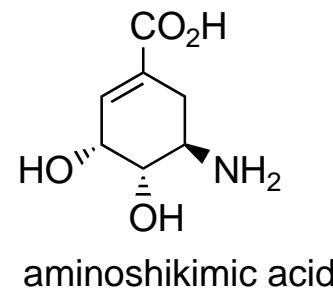
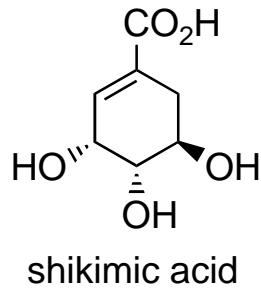
Doldouras, G. A.; Kollonitsch, J. *J. Am. Chem. Soc.* **1978**, *100*, 341-342.  
Ramamurthy, T. V.; Ravi, S.; Viswanathan, K. V. *J. Labelled Compd. Rad.* **1988**, *25*, 809-814.

## Glucose vs. Benzene; CO<sub>2</sub> vs. Petroleum

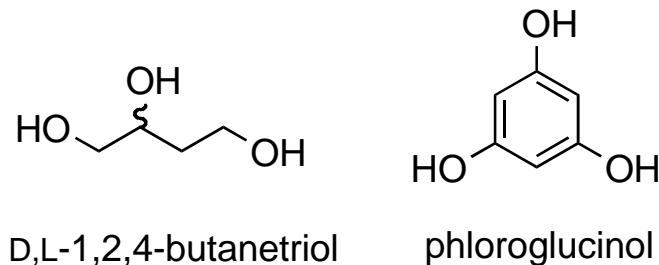




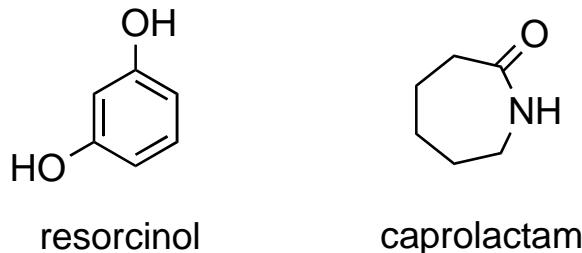
✓ Chiral Chemicals



✓ Oxygen-Rich Chemicals

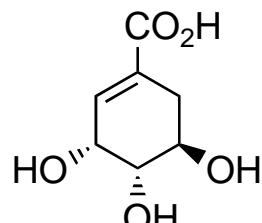


✓ Petrochemicals



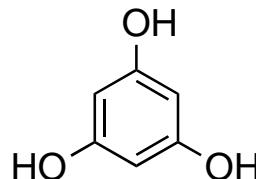


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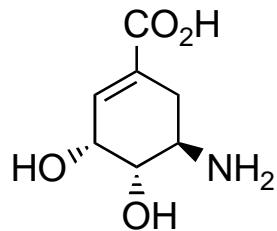
shikimic acid

NSF, NIH



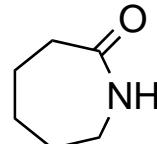
phloroglucinol

ONR



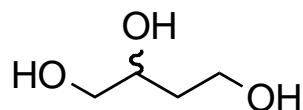
aminoshikimic acid

NIH



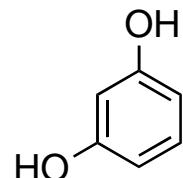
NSF

caprolactam



D,L-1,2,4-butanetriol

ONR NSF



resorcinol

NSF